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High Performance Liquid Chromatography/Video Fluorometry. Part II. Applications.

by

(10) Dennis C. Shelly, Michael P. Fogarty and Isiah M. Warner

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Department of Chemistry
Texas A&M University
College Station, Texas 77843

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High Performance Liquid Chromatography/Video Fluorometry:

Part II. Applications

Dennis C. Shelly, Michael P. Fogarty and Isiah M. Warner*

Department of Chemistry
Texas A&M University
College Station, TX. 77843

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Summary

A Video Fluorometer is used as a detector for HPLC to characterize highly complex samples. Data selection and evaluation techniques are discussed so as to speed the spectral identification process. Several well-known Polynuclear Aromatic Hydrocarbons are found in the samples.

Introduction

The role of trace organic analysis has expanded in recent years due to the importance of identifying and quantifying trace levels of organic pollutants. Our need to monitor the quality of the environment in today's industrialized society has required the development of new analytical methods, specifically designed to measure the presence of these so-called "priority pollutants" in often complex samples. In response to this challenge, high resolution separation techniques such as gas chromatography (GC), liquid chromatography (LC) and high performance thin layer chromatography (HPTLC) have developed important roles in environmental analysis. Improvements of these procedures, namely capillary GC and high performance liquid chromatography (HPLC) have brought the analyst close to "complete" separation of sample constituents. Still, the importance of single species determinations cannot be overlooked. These methods are typically very rapid and in many situations offer "state-of-the-art" in terms of specificity and sensitivity.

Modern methods of multicomponent organic analysis are characterized, not only by sophisticated separation techniques, but also equally complex detection devices, thus increasing the specificity and selectivity of the analysis and hence, the distinction of the multi-parameter approaches as "hyphenated methods" [1]. Perhaps the most important of these techniques is gas chromatography-mass spectrometry (GC-MS) [2]. This instrumentation has been employed for the identification and quantification of 22 polycyclic aromatic compounds (PNAs) in lake and river sediments, street dust and airborne particulate samples [3]. A related technique, liquid

chromatography-mass spectrometry (LC-MS),has been utilized for multi-component determinations, although on a more limited scale [4]. Imaging detectors, such as the silicon vidicon have been used to monitor the absorption [5], fluorescence [6] and combined excitation and emission spectra [7-9] of LC effluents. Conventional fluorescence detectors, of the scanning type, have also aided in the identification of chromatographic peaks [10] by the stopped-flow scanning technique. Typically, an integrated analysis scheme is used for the determination of specific organic compounds in complex samples [11].

This paper reports the application of liquid chromatography/video fluorometry (LC-VF) to the qualitative analysis of a crude oil, ash-residue sample. The focus of this work is the interpretation of data resulting from the application of techniques described earlier [9] for the rapid, expanded identification of fluorescent constituents in a very complicated matrix. The sample is somewhat unique in that it is the partially burned residue from crude oil and thus would be expected to contain many of the same compounds found in crude oil and fly ash. In this light, we can compare our results to previously reported studies of these two sample types. The nature of our data is such that an exhaustive treatment would exceed space limitations. Therefore we will limit our results to include the positive and tentative identification of "major" fluorescent components.

Experimental

Instrumentation

The LC-VF apparatus has been described previously [9] and consists of the Altex Model 312 MP Liquid Chromatograph (Altex Scientific,

Berkeley, CA), Princeton Applied Research OMA-2 Optical Multichannel Analyzer (EG&G Princeton Applied Research Corp., Princeton, NJ) and a Hewlett Packard 9845T Computer System (Hewlett Packard, Fort Collins, CO).

Chromatography

All chromatography was performed using the Altex HPLC. Two types of separations were carried out. Preparative-scale chromatography was utilized for an initial fractionation of the sample based on ring size using a 30 cm X 10 mm i.d. aminosilane column (Supelco Inc., Belfonte, PA). The pooled, concentrated fractions were then analytically separated on a 25 cm X 4.6 mm i.d. Ultrasphere ODS column (Beckman Instruments, Irvine, CA). The aminosilane column was operated in the normal phase mode using cyclohexane as the mobile phase, and at a flow rate of 2.0 mL min^{-1}. The Ultrasphere ODS column was utilized for reversed-phase separations using acetonitrile/water solvent system, under gradient elution conditions, and a flow rate of 1.0 mL min^{-1}. A MPLC Guard Column (Chromatix, Sunnyvale, CA), packed with RP-18 silica was used to protect the analytical column.

Chemicals and Reagents

Both cyclohexane and acetonitrile were distilled in glass and used as purchased from Burdick and Jackson Labs, Mushegon, MI. Type III Reagent Grade water was obtained from a Millipore, Milli Q filtration/purification system (Millipore, Bedford, MA). Certified ACS grade benzene (Fisher, Fairlawn, NJ) was used without purification.

Sample Preparation Procedure

Approximately 5 g of burned oil residue was Soxhlet extracted with benzene for 24 hours. The extract was concentrated to 0.035 g L^{-1} by evaporating to dryness and redissolving in cyclohexane. To remove particulates the extract was filtered through a 0.5 μm fluorocarbon membrane filter. A total of nine 100 μL injections were chromatographed on the amine column. The individual fractions were pooled, evaporated to dryness under dry nitrogen and redissolved each in 200 μL of acetonitrile. Each sample was again filtered, in this case using the MF-1 Centrifugal Microfilter with a 0.2 μm, pore size regenerated cellulose filter (Bioanalytical Systems, W. Lafayette, IN).

Analysis Procedure

After fractionation on the amine column, individual samples were analyzed by the LC-VF using procedures identical to the PNA standards previously described [9].

Results and Discussion

Fluorescent Data Selection

The results of the amine fractionation are shown in Figure 1. Fraction I represents one, two and some three ring PNAs. Fraction II corresponds to three and some four ring compounds. Fraction III contains four and larger ring compounds, as demonstrated by Wise et al. [10]. No attempts were made to confirm these ring fractionation assignments with standards since we have performed similar separations of shale oil [12] and found these designations to be basically correct.

The results of the reversed-phase chromatography of each of these fractions are shown in Figure 2. The Spectrum Number axis

Figure 1

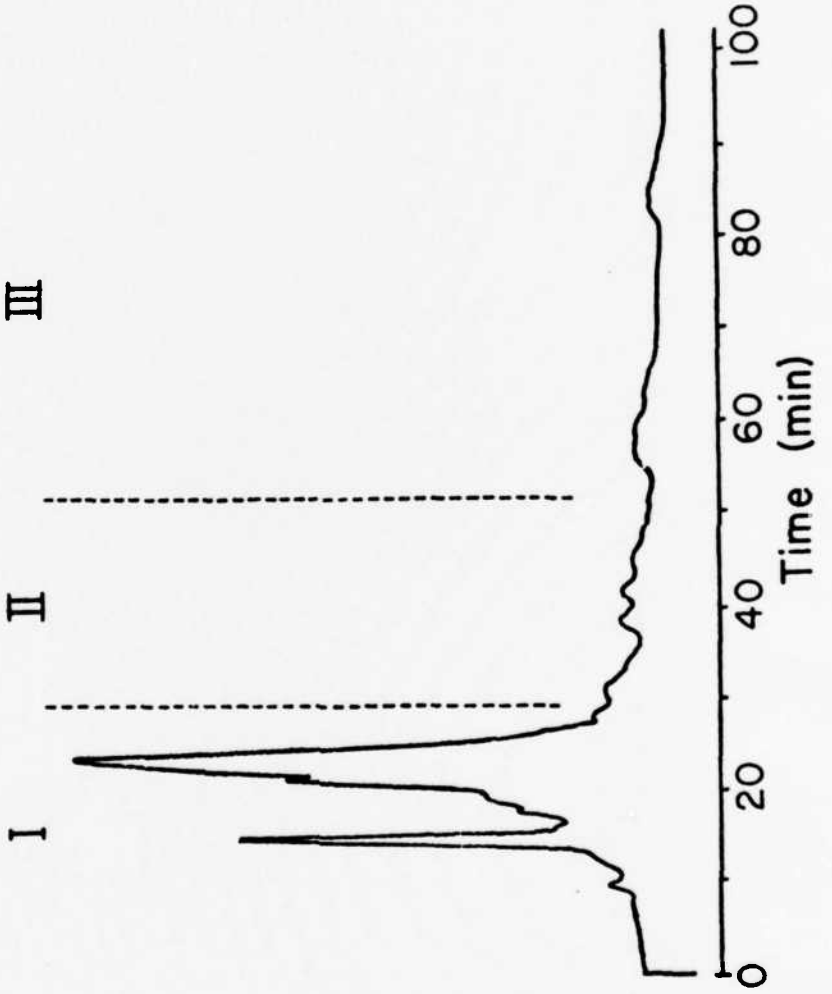
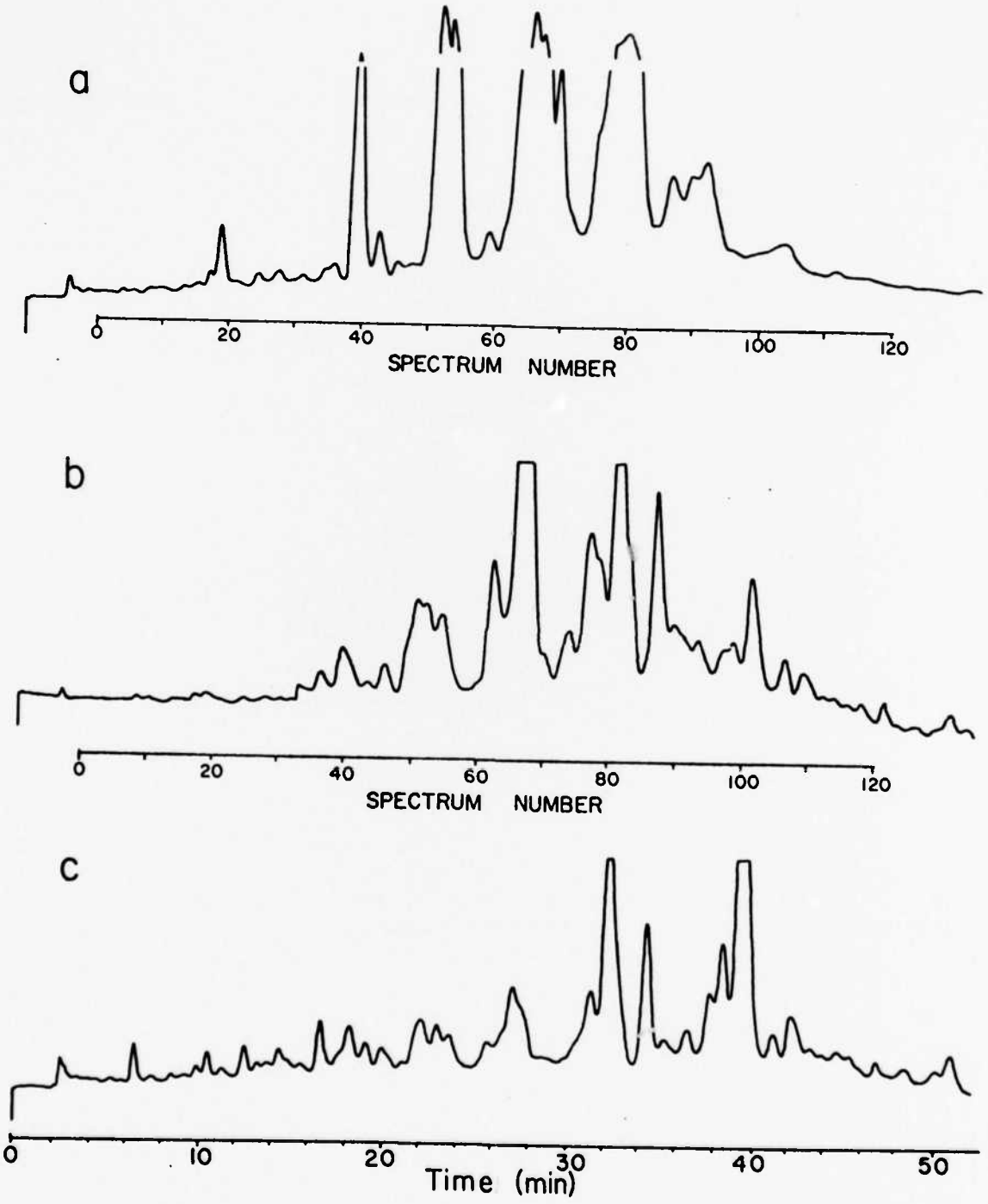


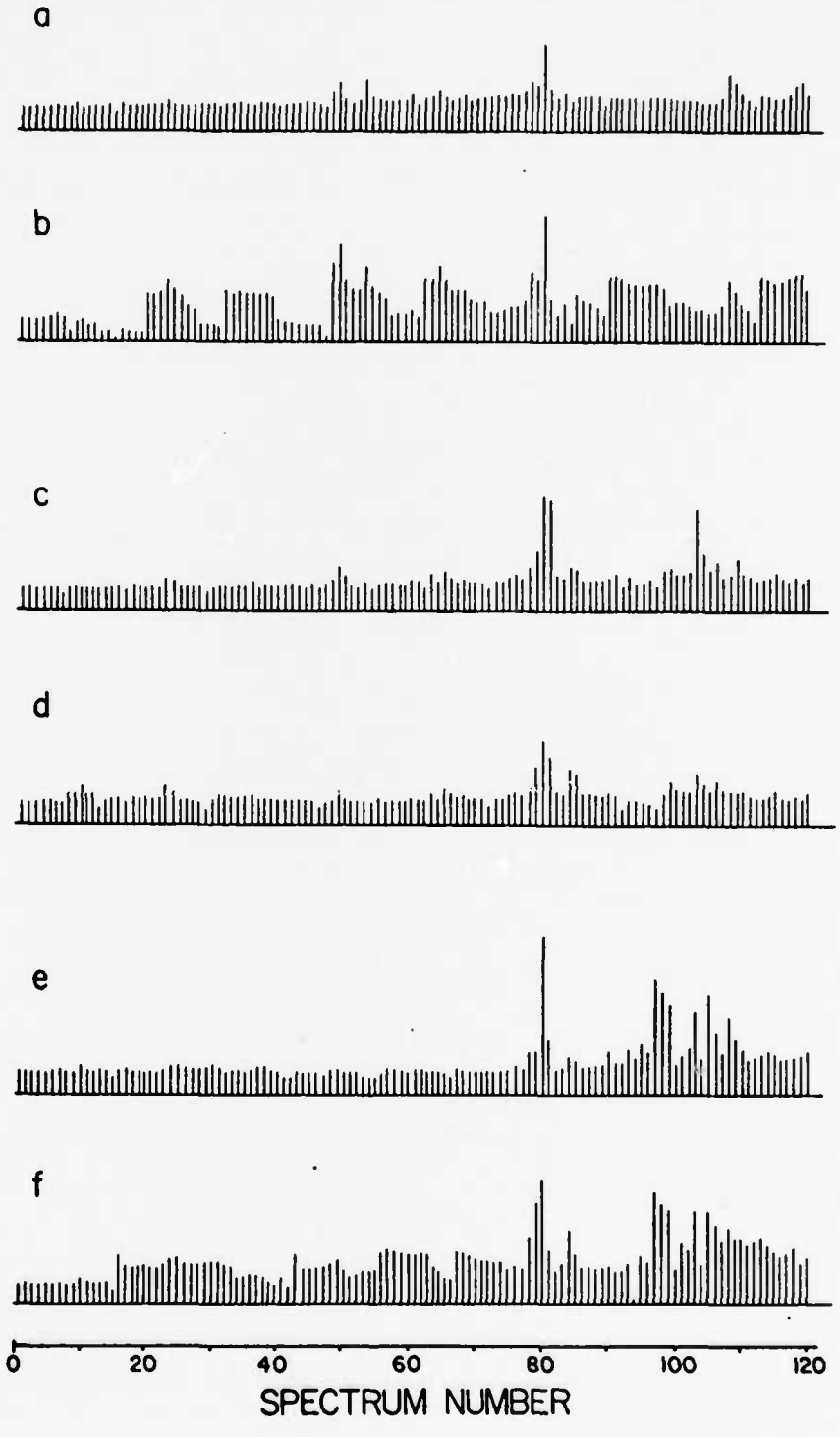
Figure 2


a
0
20
40
60
80
100
120
SPECTRUM NUMBER
b
0
20
40
60
80
100
120
SPECTRUM NUMBER
c
0
10
20
30
40
50
Time (min)

below each chromatogram indicates the time frame over which the fluorescence Emission/Excitation Matrices (EEMs) were taken. This time frame was the same for Fractions II and III (Figures 2b and 2c). The objective here was to include as much of the eluting peaks within the 120 EEM time sequence, hence the different gradient conditions. There are indeed many components indicated. However, there appears to be adequate separation. It is certainly better than if the sample had not first been fractionated. There are several areas of overlap between the fractions indicating that separation on the amine column was perhaps not as extensive as might be expected. Nevertheless, due to the extreme complexity of the Soxhlet extract and nature of the amine separation, these results are not unreasonable or deleterious. We could, at this point, make tentative peak assignments based on the number of rings expected in each fraction and the retention times of the components relative to those obtained for the PNA standards, enumerated earlier [9]. However, to realize the full potential of HPLC-VF, we shall first consider the fluorescence spectra EEMs and then confirm our findings with retention time and ring size data.

Fluorescence chromatograms of these fractions, acquired concurrently with the absorption chromatograms of Figure 2, are shown in Figure 3. During the analytical separation 120 EEMs are gathered and recorded for later examination. The fluorescence chromatograms of Figure 3 are derived from the EEM sequence data to provide an indication of the eluent fluorescence as the chromatogram developes. Each EEM represents fluorescence as a function of multiple emitting and exciting wavelengths. The wavelength ranges are 263 to 582 nm in emission and 208 to 554 nm in excitation. Since these data are in

Figure 3



matrix form, each row represents an emission spectrum and each column is an excitation spectrum, each consisting of 50 points. The total fluorescence traces in Figures 3b, 3d and 3f result from summing the fluorescence signal over the entire 2500 point matrix, thus representing all the emitting and exciting frequencies. In Figures 3a, 3c and 3e the plotted signal arises from summing over a single row within the matrix. This is equivalent to summing all the emission resulting from excitation at a single wavelength, which for Figure 3 is 314 nm exciting light. Using these techniques we can view the total fluorescence derived from either partial or total excitation in real time. From Figure 3 we note that all three fractions show a fluorescent peak at spectrum (EEM) 80. This corresponds to 32.62 minutes for Fraction I and 32.04 minutes for Fractions II and III. By examining Figure 2, we find major peak(s) in the absorption chromatograms at these times. The usefulness of total fluorescence chromatograms representing partial and total excitation wavelengths is demonstrated by the relative differences in the peak heights of the pairs of chromatograms for each fraction. For example, note the differences in the intensities for Spectrum 109 in Figures 3c and 3d. There is a definite peak in Fraction II at 42.48 minutes as seen in Figure 3c. However, we cannot make the same statement for Figure 3d, since the intensity of this peak is not significantly different from that of Spectra 108 and 110 in Figure 3d. In this case, the fluorescence from Spectrum 109 contributes more to the relative emission in the chromatogram of Figure 3c than it does to the overall signal plotted in Figure 3d. In this way, the contribution from minor components is magnified resulting in increased sensitivity

over that which would have been possible using only the total fluorescence-total excitation plots. This is important because in order to speed data analysis we must be selective by choosing spectra which contain sufficient signal for further examination and data reduction. Otherwise, the time required for these efforts will become prohibitively long compared to actual time involved in acquiring the data.

A technique which circumvents examination of individual spectra is to format the EEMs as Time-Emission and Time-Excitation arrays. Figure 4 shows Time-Emission arrays, plotted as isometric projections for the three fractions. These data can be viewed as a series of fifty chromatograms representing an equal number of emission wavelengths for which the fluorescence signal resulted from excitation between 208 and 554 nm. Although the data contains discontinuities and noise, there are several interesting results that can be cited. The data of Figure 4A shows more signal in the low emission wavelength area than Figures 4B and 4C. This is reasonable since Fraction I would be expected to contain small ring compounds, such as naphthalene, which are characterized by short wavelength emission [13]. Fraction III contains the highest density of fluorescence and also an increased amount of signal in the long wavelength portion of the emission spectrum. This also appears at high spectrum numbers indicative of relatively late elution in the chromatography. These observations are consistent with the contention that large ring PNAs, such as perylene and pentacene, are present in this fraction. These species are strongly retained on the amine column, elute late on reversed phase C_{18} and possess emission maxima in the long wavelength region.

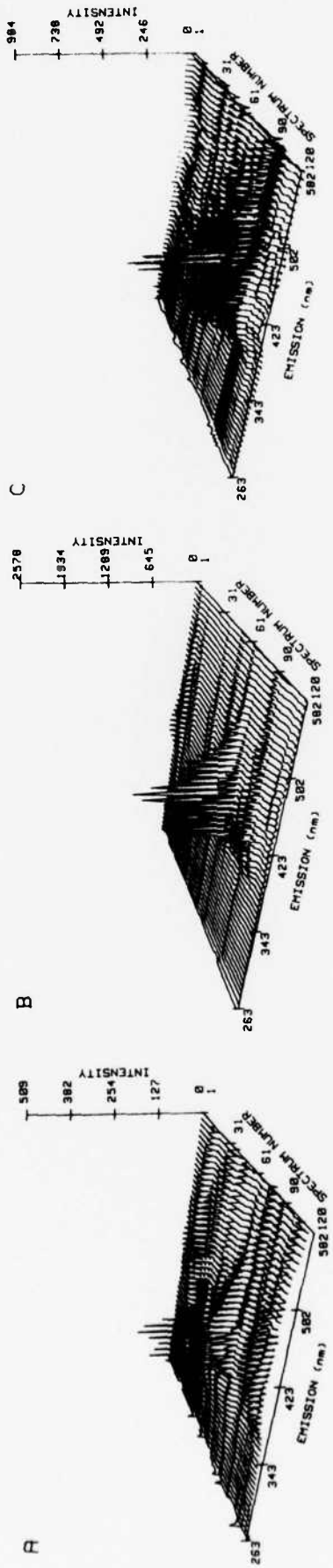

INTENSITY
984
738
492
246
0
SPECTRUM NUMBER
31
61
98
582128
EMISSION (nm)
263
343
423
582
C
INTENSITY
2578
1934
1289
645
0
SPECTRUM NUMBER
31
61
98
582128
EMISSION (nm)
263
343
423
582
B
INTENSITY
509
382
254
127
0
SPECTRUM NUMBER
31
61
98
582128
EMISSION (nm)
263
343
423
582
A

Figure 4

Using the Time-Emission data of Figure 4 and corresponding Time-Excitation arrays (not shown) we can reconstruct total fluorescence EEMs for each of the fractions. These plots are shown in Figure 5. The same observations, noted above for Figure 4, can be extended to the data in Figure 5, with respect to the spectroscopic nature of the fluorescent components.

Identification Procedure

Using the data from Figures 2, 3 and 4 it is possible to select EEMs for further analysis. Due to the great quantity of data, this "pre-screening" procedure is necessary to speed the spectral identification process. The methodology involved, here, is composed of three steps. First, the selected data are plotted as isometric projections to qualitatively determine the spectral properties and extent of contribution of individual components to the summed fluorescence of the particular fraction as in Figure 5. Next, the degree of complexity and amount of overlap between the sequence EEMs is determined and appropriate spectra are spectrally deconvoluted using routines such as Eigenanalysis [16] or Ratio [17]. Finally, the results of spectral decomposition are compared to the PNA standards, whose spectra were previously acquired and compiled in a reference library. In most cases, this consists of overlaying the excitation and emission spectra, obtained from Eigenanalysis of the unknowns, with several similarly derived spectra from the standards. Differences in spectral profile are noted, as well as are shifts in excitation and emission maxima. These observations are then correlated with published data concerning substituent effects on

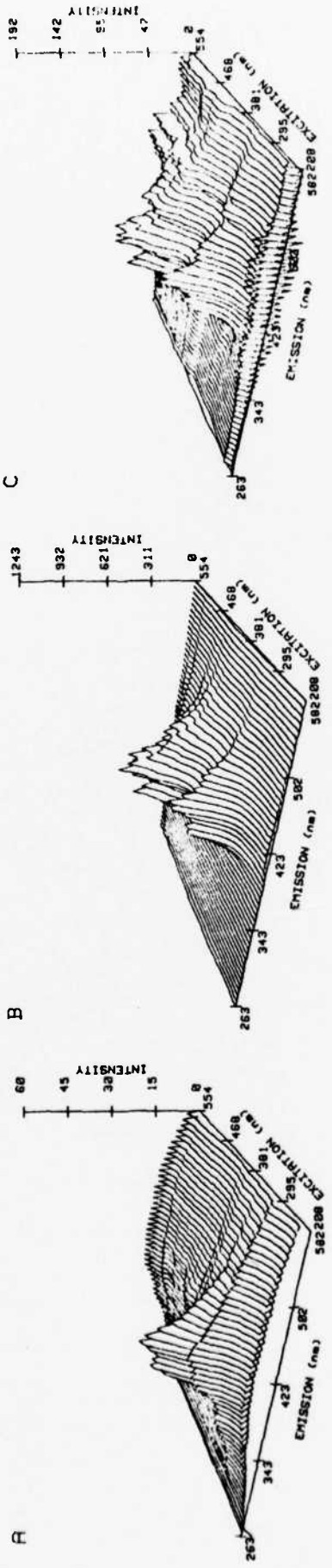


Figure 5

PNAs [13-15]. In addition, the chromatographic properties of the standards are taken into account, both from bulk fractionation on the amine column and analytical separation via reversed phase HPLC. As can be realized, there are many parameters which can be used for identification of unknown components, those obtained from spectroscopic as well as chromatographic analysis.

Spectral Deconvolution and Interpretation

Table 1 provides a summary of the results of spectral interpretation for the data from Fraction I. Similarly, Table 2 and Table 3 list the results from spectral analysis of the EEM data for Fraction II and Fraction III, respectively. Notice that each Spectrum Number listed is a corresponding peak in the fluorescence chromatograms of Figure 3. It is somewhat puzzling that benzo[a]pyrene appears in all three fractions, especially Fraction I. This seems to indicate that the initial fractionation on the amine column did not proceed as previously reported [10]. More importantly, however, this finding indicates the extent of chromatographic overlap, despite the apparent adequate resolution shown in Figure 1. The fact that benzo[a]pyrene dominates The ash extract is also demonstrated in the three spectra from Figure 5. Its presence is obvious in Figures5B and C while somewhat observed in Figure 5A by phenanthrene and anthracene derivatives. Also, evidenced in Tables 1, 2 and 3, is the occurence of several multicomponent EEMs which demonstrates that chromatographic overlap exists in even the best analytical separation.

To analyze these multicomponent EEMs two types of matrix deconvolution algorithms are used. One of these procedures involves

Table 1

Interpretation of Fluorescence Spectra from Fraction I

Spectrum #	Time	Assignment
23	11.02	Acridine Deriv. 1
49	21.46	Fluoranthene
53	22.90	Pyrene Deriv.
64	26.86	Phenanthrene Deriv. 1
68	28.30	7,8-Benzoquinoline Deriv.
78	31.90	Phenanthrene Deriv. 2 and Deriv. 3 plus Fluoranthene Deriv. 1
80	32.62	Phenanthrene Deriv. 2 and Driv. 3
83	33.70	Benzo[a]pyrene
85	34.42	Benzo[a]pyrene
108	42.70	1,2,3,4-Dibenzanthracene Deriv.
119	46.66	Chrysene Deriv. 1

Table 2

Interpretation of Fluorescence Spectra from Fraction II

Spectrum II	Time	Assignment
10	6.84	Acridine Deriv. 2
23	11.52	Acridine Deriv. 1
49	20.88	Fluoranthene
65	26.64	1,2-Benzanthracene
78	31.32	Fluoranthene Deriv. 1 Plus Perylene
79	31.68	Fluoranthene Deriv. 1, Perylene plus Anthracene Deriv. 1
80	32.04	Fluoranthene Deriv. 1, Perylene plus anthracene Deriv. 1
81	32.40	Anthracene Deriv. 1
84	33.48	Benzo[a]pyrene
99	38.88	Fluoranthene Deriv. 2 plus Anthracene Deriv. 3
103	40.32	Chrysene Deriv. 2
106	41.40	Perylene Deriv. 1
109	42.48	Chrysene Deriv. 3 plus Chrysene Deriv. 4
110	42.84	Chrysene Deriv. 4
115	44.64	Decacyclene Deriv.

Table 3

Interpretation of Fluorescence Spectra from Fraction III

Spectrum #	Time	Assignment
78	31.32	Fluoranthene Deriv. 1
79	31.68	Perylene
80	32.04	Anthracene Deriv. 1
84	33.48	Benzo[a]pyrene
97	38.16	Fluoranthene Deriv. 2
98	38.52	Fluoranthene Deriv. 2 plus Anthracene Deriv. 2
99	38.88	Anthracene Deriv. 3
103	40.32	Chrysene Deriv. 2
105	41.04	Perylene Deriv. 1
106	41.40	Benzo[g,h,i]perylene Deriv.
108	42.12	Chrysene Deriv. 3

eigenvalue decomposition of the data into eigenvectors or "pure" component spectral vectors [16]. This technique is, in most cases, limited to the presence of no more than two components due to noise and spectral overlap constraints. A more useful approach, recently developed, is a ratio method [17] which can be used for three, four and higher numbers of components. Table 4 shows the types of deconvolution strategies that were employed for the multicomponent spectra from the three fractions.

An examination of Spectrum 80 from Fraction I illustrates the use of the Eigenanalysis procedure for spectral deconvolution. Figure 6 shows the EEM of Spectrum 80 while Figure 7 shows the excitation and emission spectra resulting from a two component approximation of Spectrum 80. The spectra of Figure 7 resemble those of phenanthrene, especially the excitation profiles. While the excitation spectra are similar, the emission spectra are very different, not only in terms of structure but also with respect to emission maxima. It is very likely that two isomeric alkyl phenanthrenes could appear together in the chromatography. This reasoning is supported by the relatively low fluorescence yield for phenanthrene and the observation that alkyl substitution of similar compounds such as anthracene frequently increases the quantum efficiency of emission with a loss of structure in the emission spectrum [14,15].

If more than two components appear in the EEM, Ratio is a convenient method for spectral decomposition. The application of this technique to chromatographically unresolved fluorescence data is shown in Figures 8 and 9. As noted in Table 2, Spectra 79 and

Table 4

Summary of Spectral Deconvolution Methods Applied

Fraction #	Spectrum #	Method of Analysis
I	78	Eigenanalysis
I	80	Eigenanalysis
II	78,79 and 80	Ratio
II	99	Eigenanalysis
III	98	Eigenanalysis

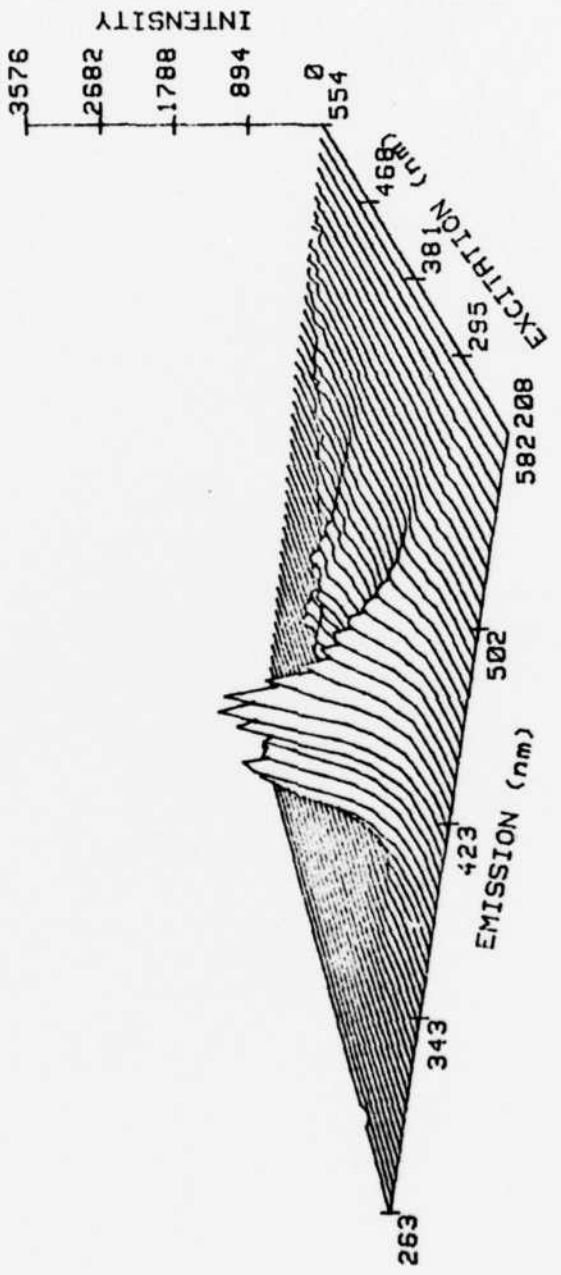
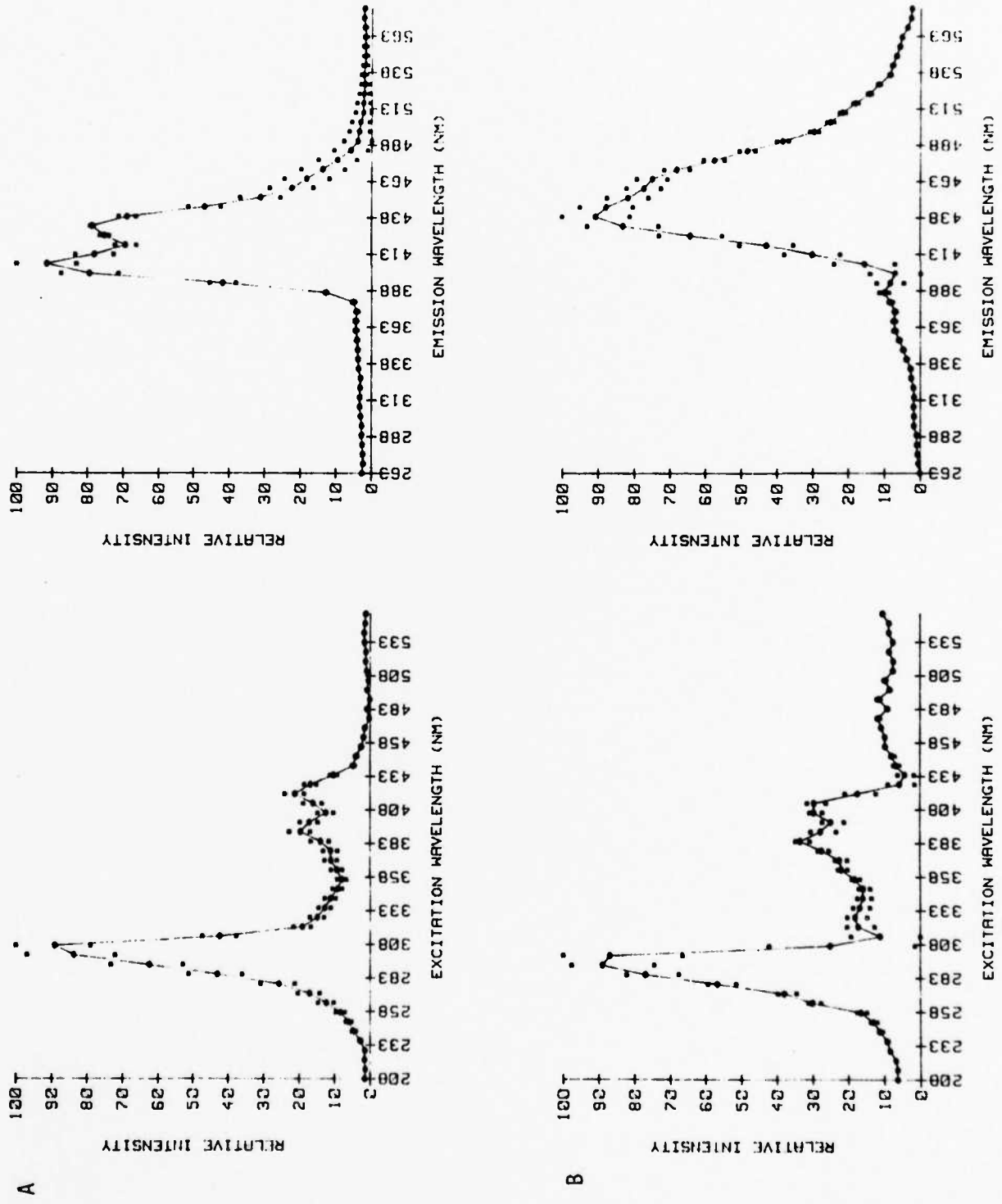

INTENSITY
3576
2682
1788
894
0
554
468
381
295
EXCITATION (nm)
582208
502
423
EMISSION (nm)
343
263

Figure 6

Figure 7


EMISSION WAVELENGTH (NM)
RELATIVE INTENSITY
EXCITATION WAVELENGTH (NM)
RELATIVE INTENSITY
A
B

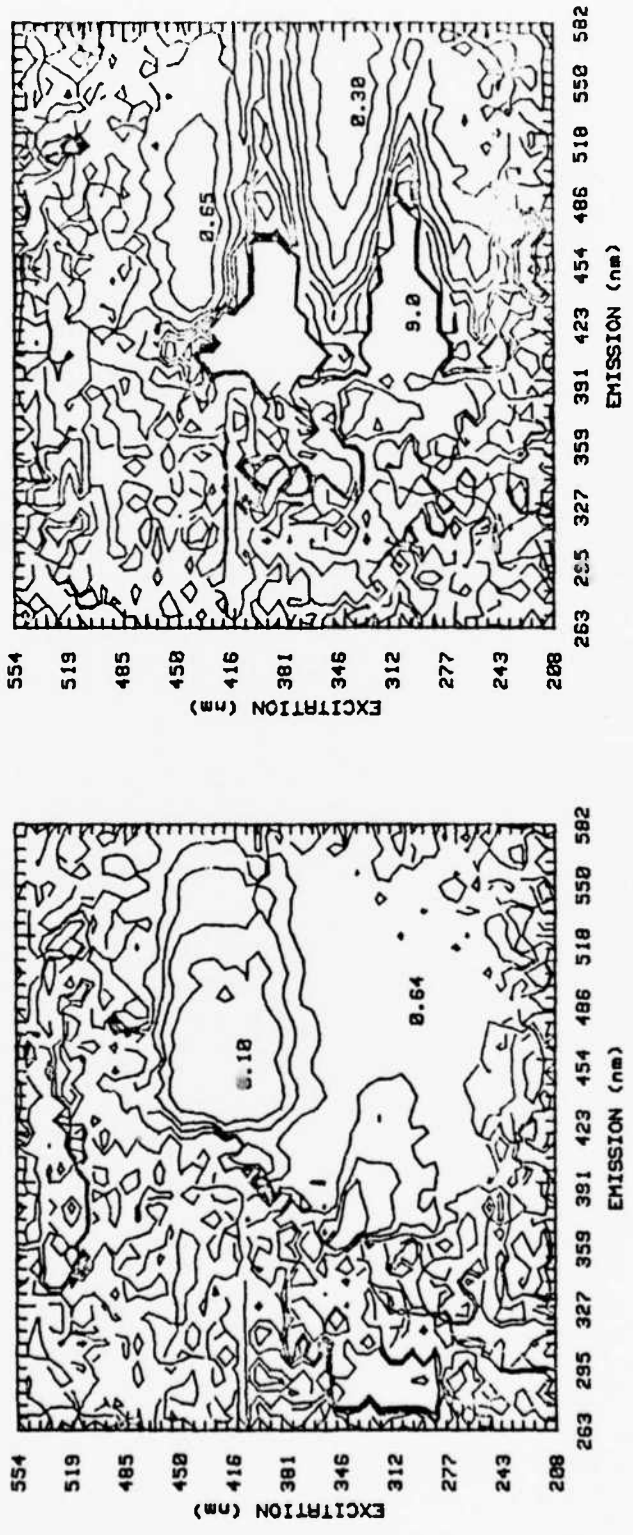

A
B
EXCITATION (nm)
EMISSION (nm)
554
519
485
450
416
381
346
312
277
243
208
263
295
327
359
391
423
454
486
518
550
582
0.18
0.64
0.65
0.38
9.0

Figure 8

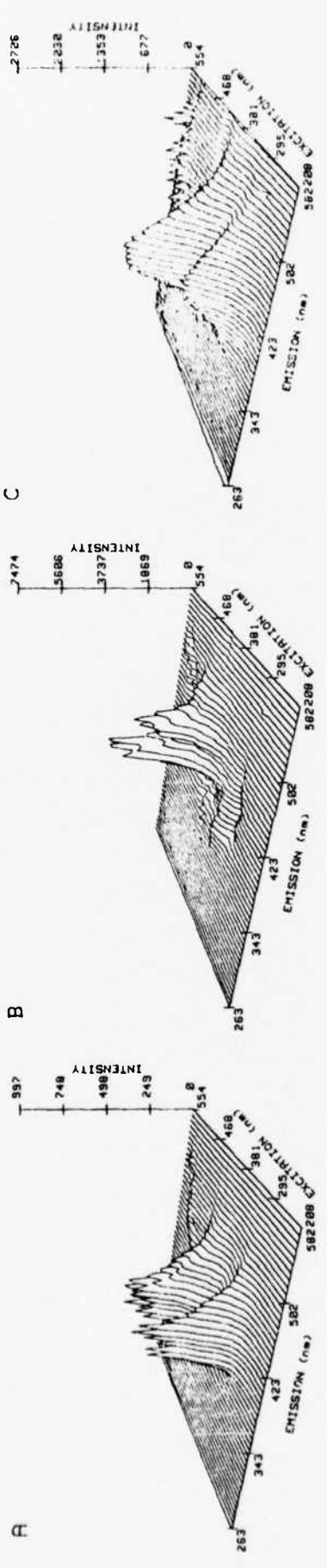
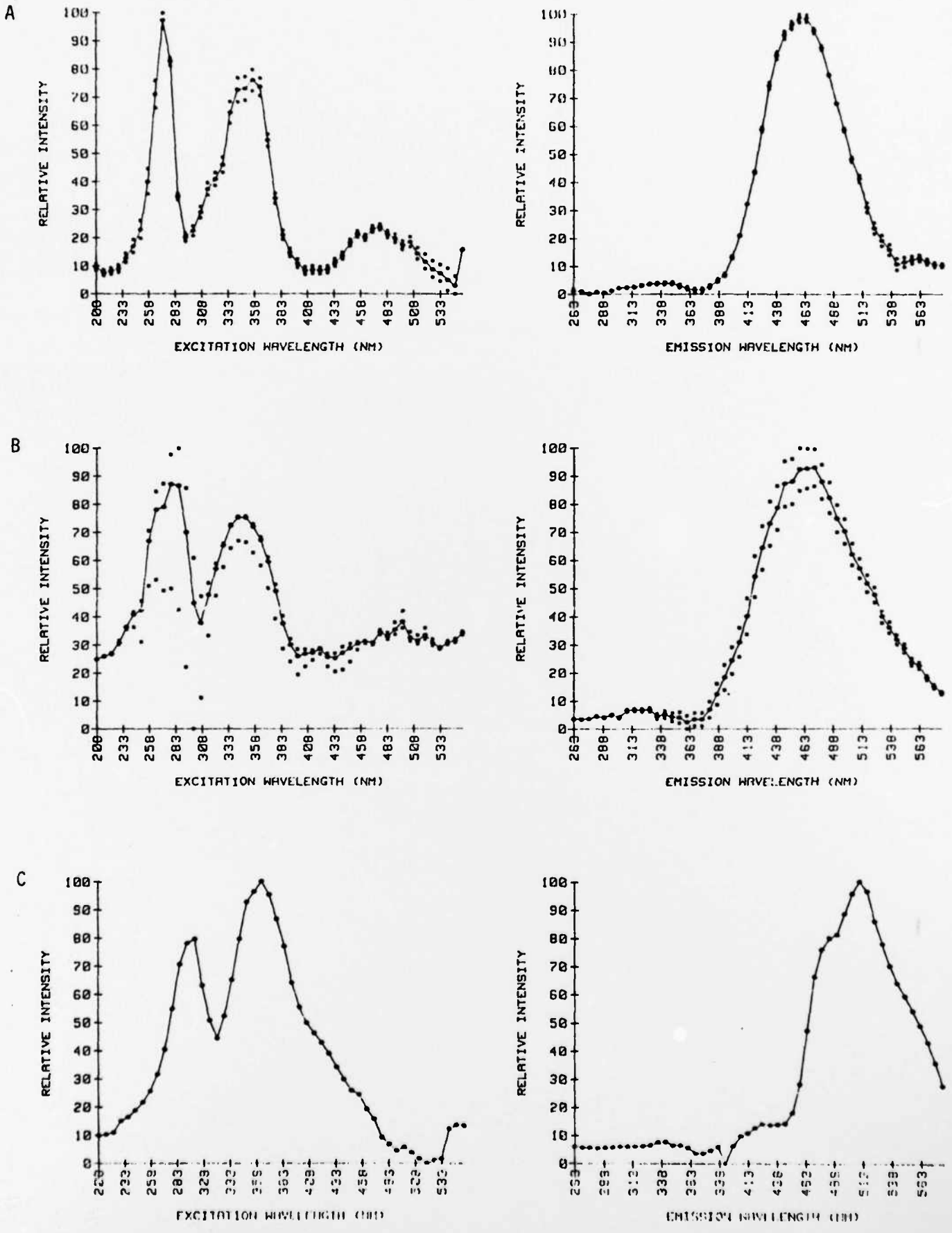


Figure 9

80 are three component and Spectrum 78 is two. After Eigenanalysis failed to properly deconvolute 79 and 80 the Ratio algorithm was used. The first step is to obtain ratio matrices which, if plotted in contour fashion, quickly indicate the number of spectral overlap areas or components. Figure 8 shows the ratio matrices of Spectrum 78 with 79 in A and Spectrum 80 with 79 in B. Notice in Figure 8B there are three plateau areas suggesting an equal number of components present in the numerator and denominator data matrices. The values of these areas are then used to enable the reduction of a single multicomponent spectrum, in this case Spectrum 79, into three single component EEMs as shown in Figure 9. Figure 9A resembles anthracene, but is more likely a derivative due to its late elution compared to anthracene. Figure 9B is an exact match, both spectrally and chromatographically with perylene. The EEM in Figure 9C is indicative of a fluoranthene derivative largely due to a displacement in the chromatogram by more than seven minutes, relative to a fluoranthene standard. Thus, Ratio is an effective means of deconvoluting multi-component, chromatographically unresolved fluorescence data.

Fluoranthenes, phenanthrenes and chrysenes are reported to be among the more abundant forms of PNAs found in crude oil [10,11]. Consistent with these findings we report the presence of three fluoranthenes, three phenanthrenes and four chrysenes, as enumerated in Tables 1, 2 and 3. Excitation and emission spectra for the fluoranthenes are shown in Figure 10 as derived from Eigenanalysis from EEMs obtained from the three fractions. In addition to the different types Figure 10B shows, basically, the same spectral

Figure 10


A
RELATIVE INTENSITY
EXCITATION WAVELENGTH (NM)
EMISSION WAVELENGTH (NM)
B
RELATIVE INTENSITY
EXCITATION WAVELENGTH (NM)
EMISSION WAVELENGTH (NM)
C
RELATIVE INTENSITY

features as the parent compound, but this data was captured almost 11 minutes later than the parent compound. Retention of spectral structure and increased chromatographic retention could indicate alkyl substitution, a situation analogous to that of anthracene. The emission spectrum of Figure 10C clearly shows increased structure relative to the previous examples. This coupled with its acquisition in the region where large ring species elute may suggest aryl substitution or bifluoranthrene. The spectra of Figure 10C show remarkable similarity to those of 3,3'-bifluoranthene [13]. The phenanthrenes are shown in Figures 7 and 11. Figure 11A shows excitation and emission spectra for the phenanthrene standard. It is with these data that the spectra in Figure 11B and Figure 7 should be compared. The species represented in Figure 11B is likely an alkyl substituted phenanthrene, since this would explain its late elution relative to the non-derivative and similar spectral properties. Perhaps the most interesting group of compounds are shown as excitation and emission spectra in Figure 12. There are four distinct chrysenes as evidenced by the different retention times and fluorescence characteristics in Figure 12. It is difficult to draw many conclusions from this data other than that Figure 12B may represent a dialkylated derivative, similar to 9,10-dimethyl-1,2-benzanthracene relative to 1,2-benzanthracene. For 1,2-benzanthracene the 9,10-dimethyl derivative elutes in the reverse order; that is, the substituted form comes before the parent compound. Since chrysene normally elutes at Spectrum 108, the species represented in Figure 12B may be of a similar type as 9,10-dimethyl-1,2-benzanthracene. The occurrence of these large ring compound in fly ash is well known [18,19]. Our samples now appear to possess chemical

Figure 11

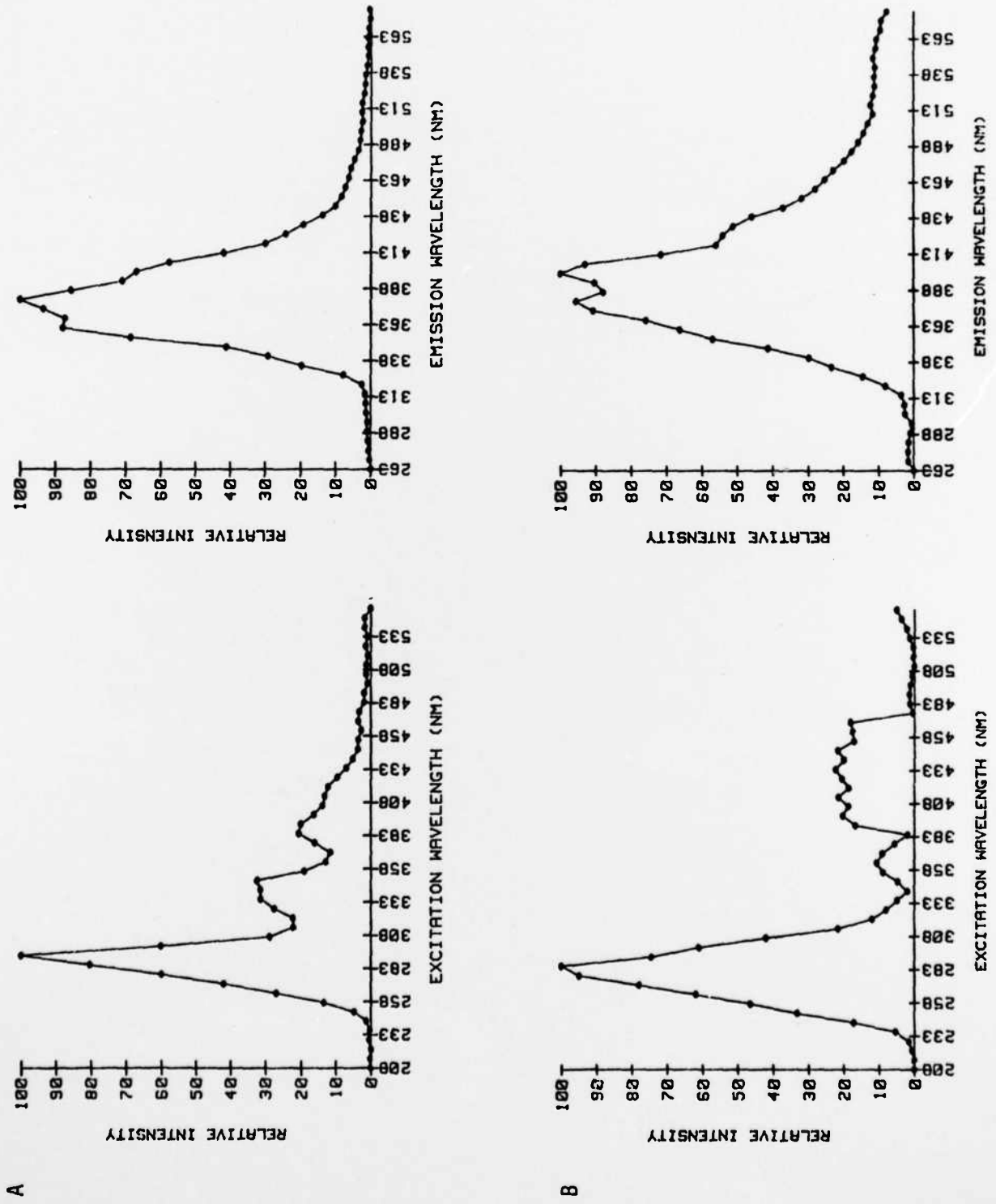
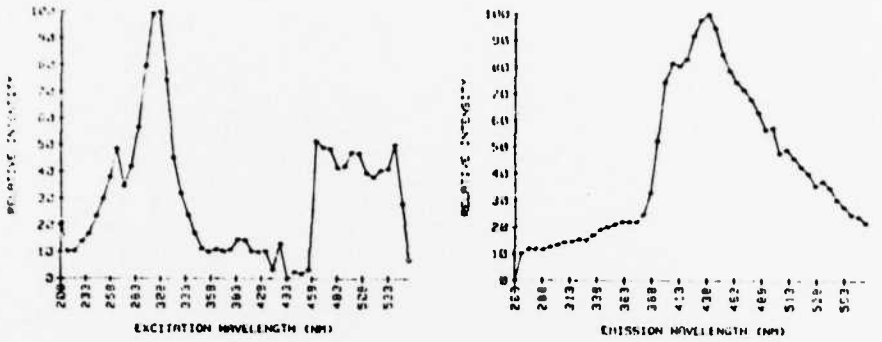
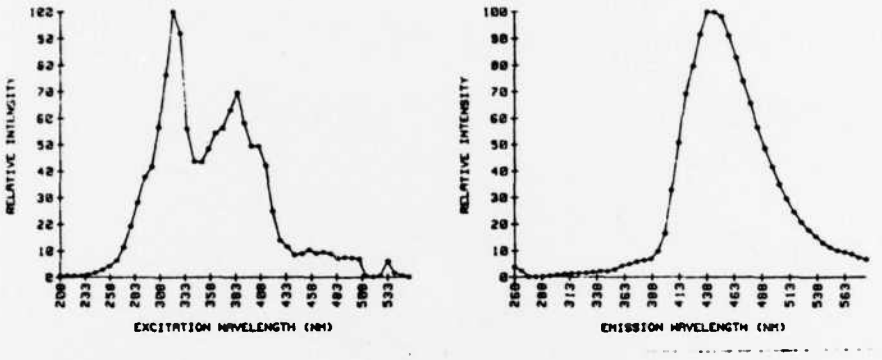

EMISSION WAVELENGTH (NM)
RELATIVE INTENSITY
EXCITATION WAVELENGTH (NM)
RELATIVE INTENSITY
A
B

Figure 12

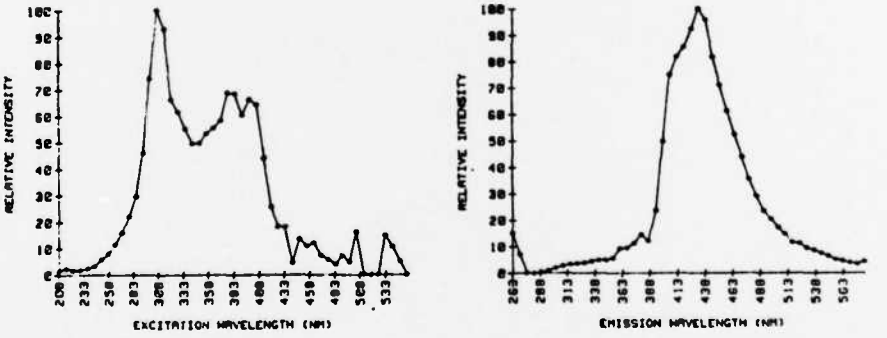
A



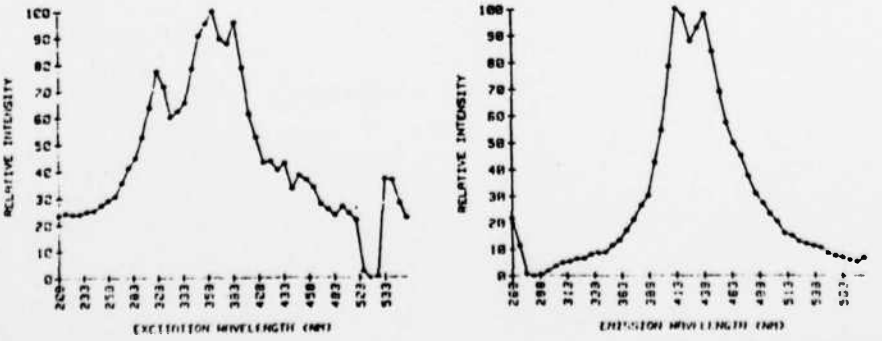
B



C



D



features of both crude oil and fly ash. Nearly all of the remaining data from Tables 1, 2 and 3 contained rather highly structured, well resolved fluorescence. For this reason as well as space limitations this data is not discussed in this manuscript.

Conclusions

The applicability of HPLC-VF for qualitative analysis of very complex samples has been explored. Attempts were made to characterize as many fluorescent components as possible by use of sophisticated data reduction techniques and correlation with standard spectra. The technique is truly multiparameter because retention time, fluorescence intensity, excitation spectrum and emission spectrum are all used to characterize fluorescent chromatographic effluents. The unique aspect of HPLC-VF is the amount and speed of data acquisition, which combined with the nature of the data provides a very powerful tool for rapid characterization of complex fluorescent mixtures. In this way HPLC-VF is a complement to GC-MS and LC-MS by providing a great deal of fluorescence spectroscopic information about individual sample constituents.

We have identified most of the major, and many of the minor, fluorescent components to conclude that our findings agree with previously published results for crude oil and fly ash samples. Benzo[a]pyrene is a major constituent of the sample as are various anthracene derivatives, fluoranthene derivatives, phenanthrene compounds and chrysene-like species. this correlates well with the reconstructed data of Figure 5. The technique is not particularly sensitive for low UV absorbing and emitting compounds such as

dibenzothiophenes and fluorenes, which are also characterized by low fluorescence efficiencies.

The future holds great promise for HPLC-VF as a powerful technique for trace organic analysis. Advances in detector technology may provide greater sensitivity so that trace and ultrace-level constituents may be detected and characterized.

Acknowledgements

This work was supported in part by grants from the Department of Energy (DE-AS05-80EV10404) and the Office of Naval Research (NR051-747). M.P.F. is also grateful for support by an American Chemical Society Analytical Division Fellowship sponsored by the Upjohn Co.

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Figure Captions

Figure 1. Fractionation of Soxhlet Extract on n-propylamine. Conditions: 100 µL sample, 2.0 mL min^{-1} flow rate and 1.28 absorbance units full scale sensitivity.

Figure 2. Analytical Reversed Phase Separations of the Sample Fractions (a) Fraction I, 50 µL injection with 30 minute linear gradient (b) Fraction II, 50 µL injection with 40 minute linear gradient (c) Fraction III, 35 µL injection with 40 minute linear gradient. Conditions: 1.0 mL min^{-1} flow rate, 65 to 90% acetonitrile in water gradient and 0.16 absorbance units full scale sensitivity.

Figure 3. Total Fluorescence Chromatograms of the Sample Fractions Fraction I: (a) fluorescence excited at 314 nm, (b) total fluorescence. Fraction II: (c) fluorescence excited at 314 nm, (d) total fluorescence. Fraction III: (e) fluorescence excited at 314 nm, (f) total fluorescence.

Figure 4. Time-Emission Arrays of the Sample Fractions (A) Fraction I, (B) Fraction II and (C) Fraction III.

Figure 5. Reconstructed EEMs from Time-Emission and Time-Excitation Arrays. (A) Fraction I, (B) Fraction II and (C) Fraction III.

Figure 6. EEM of Spectrum 80 from Fraction I.

Figure 7. Results of Eigenanalysis Deconvolution of Spectrum 80 from Fraction I. (A) Phenanthrene Deriv. 2 (B) Phenanthrene Deriv. 3.

Figure 8. Ratio contour Plots used in Ratio Deconvolution of Spectra 78, 79 and 80 from Fraction II.
(A) Ratio of Spectrum 78 with 79
(B) Ratio of Spectrum 80 with 79

Figure 9. Results of Ratio Deconvolution of Spectrum 79, Fraction II. (A) Anthracene Deriv. 1, (B) Perylene and (C) Fluoranthene Deriv. 1.

Figure 10. Excitation and Emission Spectra of the Fluoranthenes (A) Fluoranthene from Spectrum 49, Fraction I, (B) Derivative 1 from Spectrum 79, Fraction I and (C) Derivative 2 from Spectrum 97, Fraction III.

Figure 11. Excitation and Emission Spectra of Phenanthrenes (A) Phenanthrene standard (B) Derivative 1 from Spectrum 64, Fraction I.

Figure 12. Excitation and Emission Spectra of the Chrysenes (A) Derivative 1 from Spectrum 119, Fraction I; (B) Derivative 2 from Spectrum 103, Fraction II; (C) Derivative 3 from Spectrum 109, Fraction II; (D) Derivative 4 from Spectrum 110, Fraction II.

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